Result:

Constructed and verified expression vectors listed in the Lab Archive page, designated pET22b(+)_x1, pET22b(+)_x2, pET22b(+)_ArfB and pET22b(+)_ManA1.

Procedure:

1. **Design primers and entrusted Shanghai Sunny Biotechnology company to synthesize them, primers shown as Table 1**

Primer name	Primer sequence
X+	CGGAATTCCATGAATAAATTCTTAAACAAAAAAT
X1-	CCGCTCGAGTTATTCCCGCAAC
X2-	CGCTCGAGTTCCCGCAACC
M+	CGGGATCCGATGAACAGGCTTAATATCAAAAT
M-	CCGCTCGAGCTATTTTCCGCGATATTTTTT
A+	TAAATCAGTCAACCGTAATGCTG
A-	TCAAGGAGGCTTAGGATTATGG

For pET22b(+)_x1, use X+ and X1-
For pET22b(+)_x2, use X+ and X2-
For pET22b(+)_ArfB, use A+ and A-
For pET22b(+)_ManA1, use M+ and M-

2. **For construction of pET22b(+)_ArfB, we used following procedure:**

2.1 PCR to amplify arfB

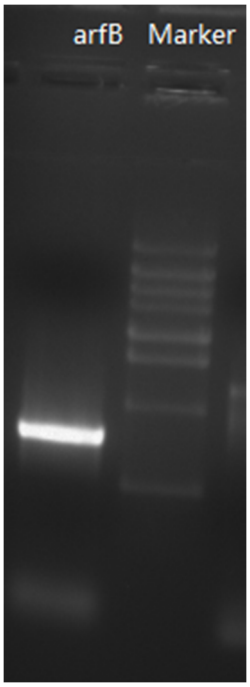
Recipe:

	arfB
Mastermix	12.5
Template(C.st)	1
Primer+ (A+)	1
Primer- (A-)	1
H_2O	10

Program:

Temperature (℃)	Time (s)
98	20*60
94	30
51	30
72	120
Goto 2	Repeat 34
72	10*60
10	hold

2.2 DNA agarose gel electrophoresis (1% agarose gel concentration)



2.3 Perform DNA gel extraction and measure concentration by Nanodrop 2000c

Sample ID	Nucleic Acid Conc.	Unit	A260	A280	260/280	260/230
arfB	13.6	ng/μl	0.273	0.14	1.94	1.37

2.4 Ligate ArfB with pMD-18 T-vector

Recipe:

Solution I	6
ArfB	5

pMD18-T	1

Incubate at 16℃ for 30min

2.5 Transform to competent E.coli (DH5α), spread100μl transformed culture to X-gal and IPTG treated Ampicilin added LB dish, incubate at 37℃ overnight

2.6 Inoculate colonies for colony PCR, positive results send for sequencing, ArfB-T1 is the sample ID of a positive sample.

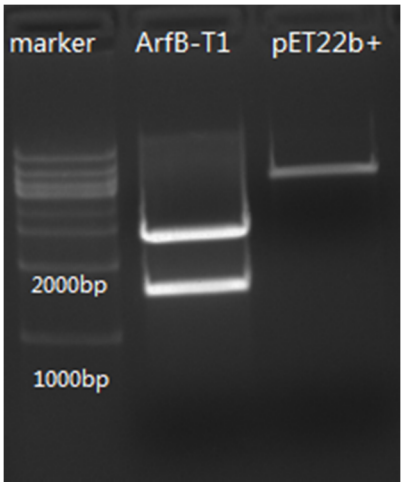
2.7 Double digest with enzymes from NEB

Recipe:

	pET22b+	ArfB-T1
SalI	1	1
BamHI	1	1
Substrate	6	2
NEBuffer 3	5	5
H_2O	37	41

Incubate at 37℃ for 8 hours, heat inactivate at 65℃ for 20 min

2.8 DNA electrophoresis (1% agarose gel concentration)



2.9 DNA Gel extraction, measure concentration with Nanodrop 2000c

Sample ID	Nucleic Acid Conc.	Unit	A260	A280	260/280	260/230
pET22b+(BamHI & SalI)	4.7	ng/μl	0.094	0.048	1.95	0.59
ArfB-T1(BamHI & SalI)	8.1	ng/μl	0.163	0.091	1.79	0.63

2.10 Ligation with T4 DNA ligase, product of NEB

Recipe:

Ligase buffer	5
ligase	1

ArfB-T1	10
pET22b+	10
H2O	24

Incubate at 16℃ for 4h

2.11 Transform ligation product to E.coli (DH5α), Spread to ampicillin loaded LB plate, culture at 37℃ overnight.

2.12 Inoculate positive clones to LB medium, send bacteria culture for sequencing.

3. For construction of pET22b (+) _X1 and pET22b (+) _X2, we used following procedure:

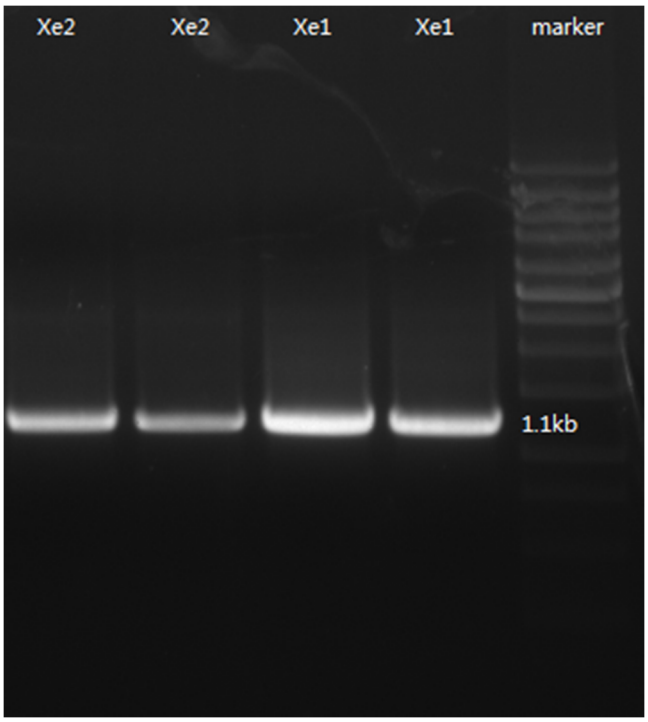
3.1PCR to amplify:
Xe1 for express XynB without His-tag
Xe2 for express XynB with His-tag
Recipe:

	Xe1	Xe2
H2O	20	20
Primer+	1	1
Primer-	1	1
MasterMix	25	25
template	2	2

Program:

Temperature(℃)		Time(s)
Xe1	Xe2	
95	95	5*60
95	95	30
54	54.9	40
72	72	120
Goto 2	Goto 2	30 times
72	72	10*60

3.2 DNA gel electrophoresis (1% agarose gel concentration)



3.3 DNA gel extraction and measure concentration with Nanodrop 2000c

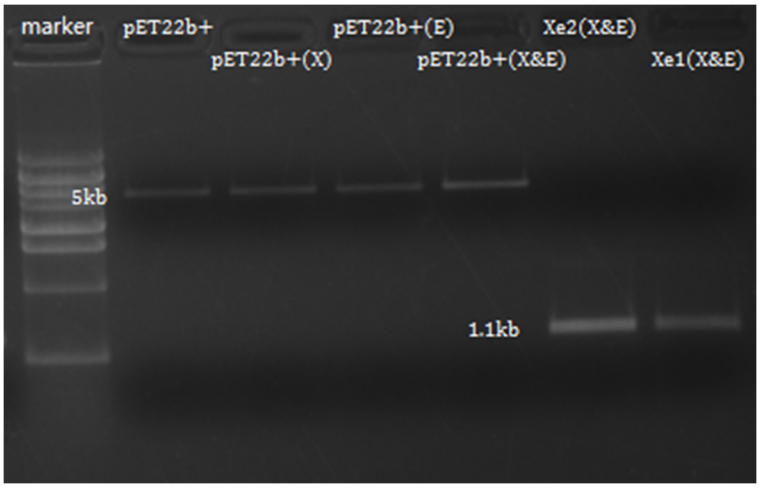
Sample ID	Nucleic Acid Conc.	Unit	A260	A280	260/280	260/230
Xe1	38.3	ng/μl	0.767	0.41	1.87	1.83
Xe1	40.2	ng/μl	0.805	0.437	1.84	1.31
Xe2	17.5	ng/μl	0.35	0.195	1.79	1.21
Xe2	16.2	ng/μl	0.323	0.168	1.93	0.18

3.4 Double digestion

	pET22b+	pET22b+(XhoI)	pET22b+(EcoRI)	pET22b+(XhoI & EcoRI)	Xe1	Xe2
XhoI	0	1	0	1	1	1
EcoRI	0	0	1	1	1	1
Buffer3.1	5	5	5	5	5	5
DNA	4(60ng/μl)	4	4	4	5	14
H2O	41	40	40	39	38	29

Incubate at 37℃ for 8 hours, heat inactivate at 65℃ for 20 min

3.5 DNA electrophoresis(1% agarose gel concentration)



3.6 DNA gel extraction and measure concentration with Nanodrop 2000c

Sample ID	Nucleic Acid Conc.	Unit	A260	A280	260/280	260/230
Xe1	2.8	ng/μl	0.056	0.032	1.75	0.21
pET22b(+)	1.8	ng/μl	0.037	0.008	4.47	0.31
Xe2	3.1	ng/μl	0.062	0.024	2.54	0.38

3.7 Ligate DNA gel extraction products

Recipe:

	Xe1	Xe2
Ligase buffer	5	5
Insert DNA	20	20
Double digested pET22b(+)	10	10
ligase	1	1
H2O	14	14

Incubate at 16℃ for 6 hours

3.8 Transform all ligation product to E.coli (DH5α), add 500μl LB, culture at 37℃, 180rpm for 30min

Spread all transformation product to ampicillin loaded LB plate, culture at 37℃ overnight

3.9 inoculate positive clones for colony PCR, PCR positive cultures send for sequencing.

4. For construction of pET22b (+) _ManA1, we used following procedure:

4.1 PCR

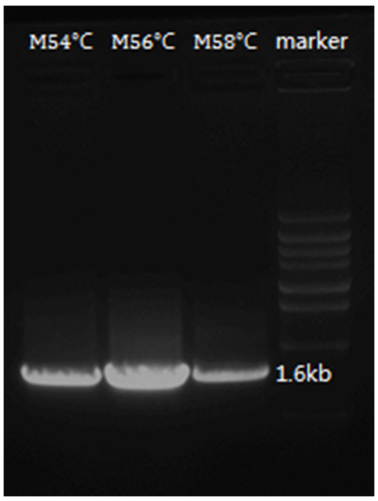
H2O	9.5
Primer（+-）	2

MasterMix	12.5
template	1

Program:

Temperature(℃)			Time(s)
94	94	94	10*60
94	94	94	30
54	56	58	45
72	72	72	120
Goto 2	Goto 2	Goto 2	30 times
72	72	72	10*60

4.2 DNA electrophoresis(1% agarose gel concentration)



4.3 Extract DNA from gel, Measure concentration with Nanodrop 2000c

Sample ID	Nucleic Acid Conc.	Unit	A260	A280	260/280	260/230
ManA1	69.3	ng/μl	1.386	0.747	1.86	1.6
ManA1	64.2	ng/μl	1.285	0.693	1.85	2.09

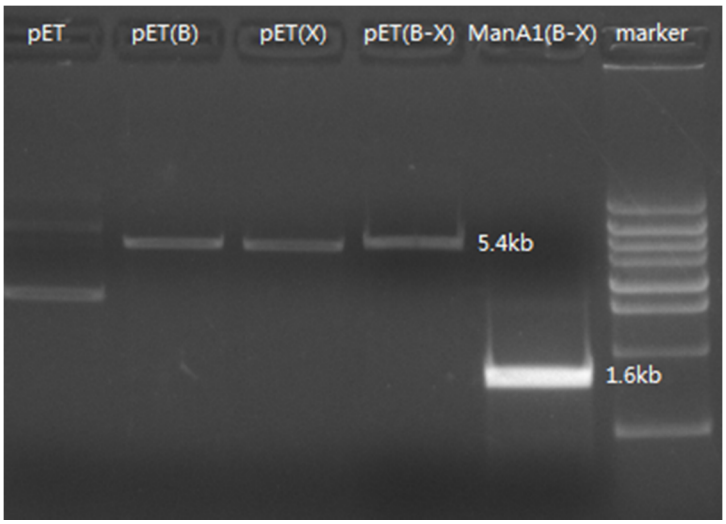
4.4 Double digestion

Double digestion（BamHI xhoI） product electro:

	ManA1	pET22b+
BamHI-HF	1	1
XhoI	1	1
Buffer4	5	5
H2O	33	35
DNA	10	4

37℃ incubate for 30 min

4.5 DNA electrophoresis(1% agarose gel concentration)



4.6 DNA gel extraction, Measure concentration with Nanodrop 2000c

Sample ID	Nucleic Acid Conc.	Unit	A260	A280	260/280	260/230
pET(B-X)	2.5	ng/μl	0.051	0.016	3.17	0.56
ManA1(B-X)	10	ng/μl	0.199	0.096	2.07	0.59

4.7 Ligation：

pET22b+（B-X）	20
ManA1(B-X)	5
Ligase buffer	5
T4 ligase	1
H2O	19

Incubate at 16℃ overnight

4.8 Transform all ligation product to E.coli (DH5α), add 500μl LB, culture at 37℃, 180rpm for 30min

Spread all transformation product to ampicillin loaded LB plate, culture at 37℃ overnight

4.9 inoculate positive clones for colony PCR, PCR positive cultures send for sequencing.